

CLAIMS

1. A method of analysis of DNA sequence, which comprises degrading, by pyrophosphatase, pyrophosphoric acid contained in a reagent used for extension reaction
5 of a DNA primer hybridized to a target nucleic acid through a complementary strand and/or degrading, by apyrase, adenosine 5'-triphosphate contained in the reagent;

conducting the extension reaction, and
10 detecting pyrophosphoric acid generated by the extension reaction.

2. A method of analysis of DNA sequence according to Claim 1, wherein the pyrophosphatase and/or the apyrase has been immobilized on a solid.

15 3. A method of analysis of DNA sequence, which comprises adding pyrophosphatase to one or more solutions which contain different deoxynucleotides, respectively, or to one or more solutions which contain different deoxynucleotides, respectively, at least one
20 of which is an analogue thereof, thereby degrading pyrophosphoric acid contained in the solutions, and

extending a DNA primer, which has been hybridized to a target nucleic acid via a complementary strand, by using the DNA primer, DNA polymerase and at
25 least one of the solutions obtained in said step and detecting pyrophosphoric acid thus generated by the extension reaction by chemiluminescence-reaction.

4. A method of analysis of DNA sequence, which

comprises adding pyrophosphatase to one or more solutions which contain different deoxynucleotides, respectively, or to one or more solutions which contain different deoxynucleotides, respectively, at least one of which is an analogue thereof, thereby degrading pyrophosphoric acid contained in the solutions, and

extending a DNA primer, which has been hybridized to a target nucleic acid via a complementary strand, by using the DNA primer, DNA polymerase and at least one of the solutions obtained in said step, converting pyrophosphoric acid thus generated by the extension reaction into adenosine 5'-triphosphate in the presence of adenosine 5'-phosphosulfate and ATP sulfurylase, and detecting luminescence caused by chemiluminescence-reaction containing the adenosine 5'-triphosphate, a luminescence-enzyme and a luminescence substrate.

5. A method of analysis of DNA sequence according to Claim 4, further comprising, after the first step, a step of removing or inactivating the pyrophosphatase in each of the solutions.

6. A method of analysis of DNA sequence according to Claim 4, wherein the first step comprises adding the pyrophosphatase to at least one of the DNA-primer-containing solution, the DNA-polymerase-containing solution, the luminescence-enzyme-containing solution, the luminescence-substrate-containing solution, the adenosine 5'-phosphosulfate-containing

208079 7128001
solution and the ATP-sulfurylase-containing solution,
thereby degrading the pyrophosphoric acid contained in
at least one of said solutions, and/or adding apyrase
to degrade adenosine 5'-triphosphate contained in at
least one of said solutions.

7. A method of analysis of DNA sequence
according to Claim 6, further comprising removing or
inactivating the pyrophosphatase and/or apyrase
contained in the pyrophosphatase- and/or apyrase-added
solution.

8. A method of analysis of DNA sequence
according to Claim 7, wherein the pyrophosphatase
and/or apyrase has been immobilized on a solid.

9. A method of analysis of DNA sequence
according to Claim 4, wherein the base at the
3'terminus of the primer is complementary to the base
one base behind the 3'terminus site of single
nucleotide polymorphism of the target nucleic acid.

10. A method of analysis of DNA sequence
according to Claim 4, wherein the second or third base
from the 3'terminus of the DNA primer has been
substituted with a base not complementary to the base
sequence of the target nucleic acid.

11. A method of analysis of DNA sequence,
which comprises:

a first step of adding pyrophosphatase to each
of a solution containing deoxyadenosine 5'- α -
thiotriphosphate, a solution containing deoxythymidine

5'-triphosphate, a solution containing deoxyguanosine 5'-triphosphate and a solution containing deoxycytidine 5'-triphosphate, thereby degrading pyrophosphoric acid contained in each of the solutions;

5 a second step of removing or inactivating the pyrophosphatase in each of the solutions, and

 a third step of extending a DNA primer, which has been hybridized to a target nucleic acid via a complementary strand, by using the DNA primer, DNA
10 polymerase and at least one of the solutions obtained in said second step, converting pyrophosphoric acid thus generated by the extension reaction into adenosine 5'-triphosphate in the presence of adenosine 5'-phosphosulfate and ATP sulfurylase, and detecting
15 luminescence caused by chemiluminescence-reaction containing the adenosine 5'-triphosphate, luciferase and luciferin.

12. A method of analysis of DNA sequence, which comprises:

20 a first step of adding pyrophosphatase to a solution containing deoxyadenosine 5'- α -thiotriphosphate, deoxythymidine 5'-triphosphate, deoxyguanosine 5'-triphosphate and deoxycytidine 5'-triphosphate, thereby degrading the pyrophosphoric acid
25 contained in the solution;

 a second step of removing or inactivating the pyrophosphatase in each of the solutions, and

 a third step of extending a DNA primer, which

has been hybridized to a target nucleic acid via a complementary strand, by using the DNA primer, DNA polymerase and at least one of the solutions obtained in said second step, converting pyrophosphoric acid thus generated by the extension reaction into adenosine 5'-triphosphate in the presence of adenosine 5'-phosphosulfate and ATP sulfurylase, and detecting luminescence caused by chemiluminescence-reaction containing the adenosine 5'-triphosphate, luciferase and luciferin.

13. A method of analysis of DNA sequence according to Claim 12, wherein the second or third base from the 3'terminus of the DNA primer has been substituted by a base not complementary to the base sequence of the target nucleic acid.

14. A method of analysis of DNA sequence according to Claim 12, wherein the extension reaction is conducted by degrading the strand, which has been extended by the extension reaction, from the 5'terminus thereof by the 5' → 3' exonuclease reaction and repeating complementary strand hybridization of the DNA primer to the target nucleic acid.